

# **Stoichiometric Constraints-Based Modeling of Large-Scale Complex Biochemical Networks**

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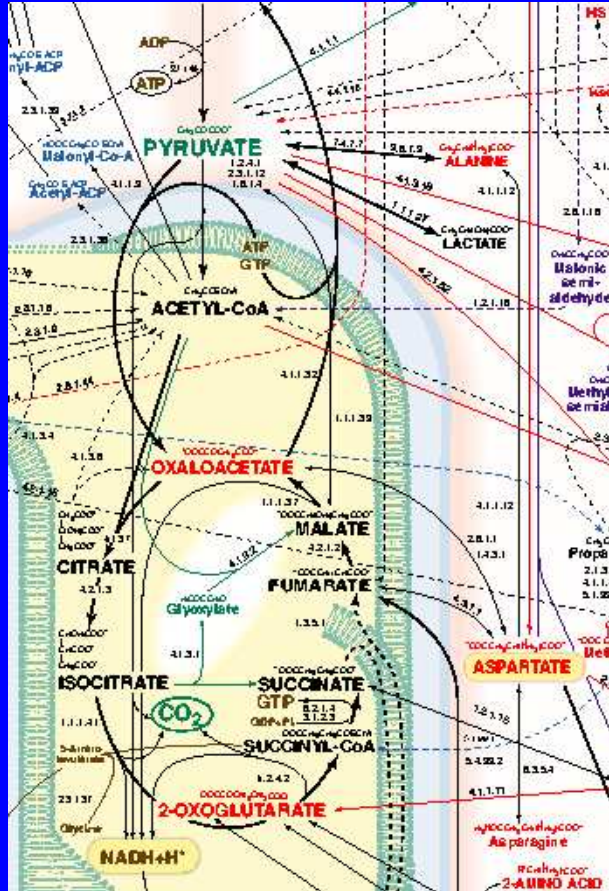
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# Abstract

Stoichiometric constraints-based models are an alternative to kinetic models when analyzing large-scale networks. Two analytic tools used in this type of modeling are Flux Balance Analysis (FBA) and Energy Balance Analysis (EBA), which use mass and energy balance constraints, respectively, to study the steady-state behavior of reaction networks. Such analysis has proven useful and successful, for example, when modeling cellular metabolism.

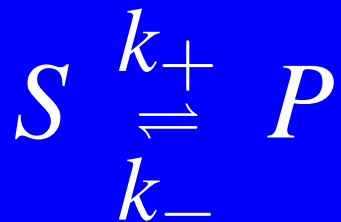
# Large-Scale Complex Networks



Sigma

- A reaction network is typically very complex.
- Reaction rate constants are usually all unknown.
- ODE's describing the kinetics contain nonlinear terms.
- Unable to find analytic solutions.

# Elementary Chemical Reaction

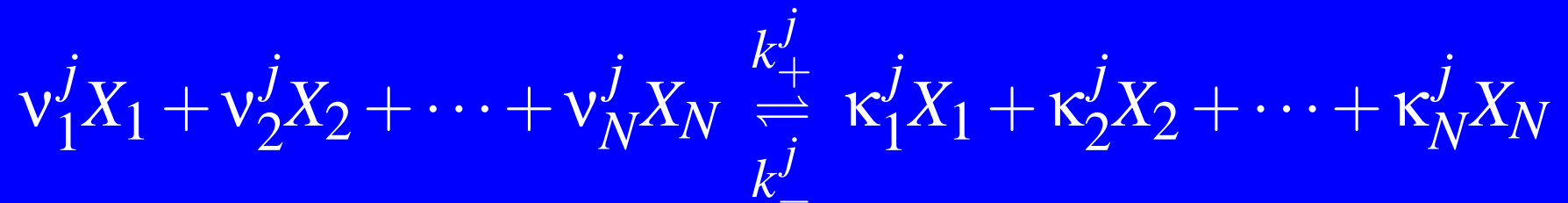


$$\frac{ds}{dt} = k_- p - k_+ s$$

$$\frac{dp}{dt} = k_+ s - k_- p$$

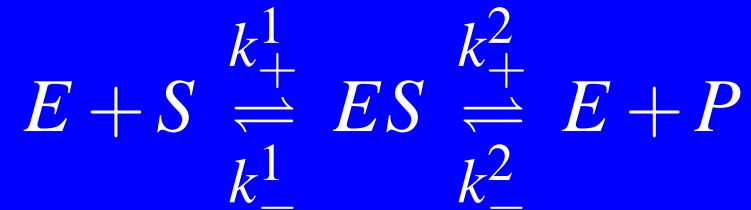
# General Chemical Reaction

If we have M reactions involving N species:



$$\frac{dx_i}{dt} = \sum_{j=1}^M (\kappa_i^j - \nu_i^j) (k_+^j x_1^{\nu_1^j} x_2^{\nu_2^j} \cdots x_N^{\nu_N^j} - k_-^j x_1^{\kappa_1^j} x_2^{\kappa_2^j} \cdots x_N^{\kappa_N^j})$$

# Enzyme Driven Reaction Kinetics



First step is rapid. Enzyme forms an intermediate complex which changes slowly relative to the rate of change of  $s$  and  $p$ . So we have coupled differential equations:

$$\frac{d(s)}{dt} = k_{-}^1(es) - k_{+}^1(e)(s)$$

$$\frac{d(es)}{dt} = k_{+}^1(e)(s) + k_{-}^2(e)(p) - k_{-}^1(es) - k_{+}^2(es)$$

$$\frac{d(p)}{dt} = k_{+}^2(es) - k_{-}^2(e)(p)$$

$$\frac{d(es)}{dt} \approx 0$$

$$\frac{d(es)}{dt} = k_{+}^1(e)(s) + k_{-}^2(e)(p) - k_{-}^1(es) - k_{+}^2(es) = 0$$

$$(es) = \frac{k_{+}^1(e)(s) + k_{-}^2(e)(p)}{k_{-}^1 + k_{+}^2}$$

$$K_{M,S} = \frac{k_{-}^1 + k_{+}^2}{k_{+}^1} \quad \text{and} \quad K_{M,P} = \frac{k_{-}^1 + k_{+}^2}{k_{-}^2}$$

$$(es) = \frac{(e)(s)}{K_{M,S}} + \frac{(e)(p)}{K_{M,P}}$$

If we assume  $S$  is continuously supplied to the system and  $P$  is continuously taken out of the system, each at some rate  $J$ , then we arrive at steady-state when:

$$J = \frac{(e_o)(k_+^1(s) - \frac{k_-^1(s)}{K_{M,S}} - \frac{k_-^1(p)}{K_{M,P}})}{1 + \frac{(s)}{K_{M,S}} + \frac{(p)}{K_{M,P}}}$$

where  $(e_o) = (e) + (es)$  is the total concentration of enzyme (free and bound).

Note that if  $J = 0$  this method predicts chemical equilibrium when:

$$\frac{(p)}{(s)} = K_{eq} = \frac{k_+^1 k_+^2}{k_-^1 k_-^2}$$



# The Stoichiometry-Based Model

Instead of representing the ODE's as:

$$\frac{dx_i}{dt} = \sum_{j=1}^M (\kappa_i^j - \nu_i^j) (k_+^j x_1^{\nu_1^j} x_2^{\nu_2^j} \dots x_N^{\nu_N^j} - k_-^j x_1^{\kappa_1^j} x_2^{\kappa_2^j} \dots x_N^{\kappa_N^j})$$

We can simplify our model to:

$$\frac{dx_i}{dt} = \sum_{j=1}^M S_i^j J^j$$

where  $\mathbf{S}$  is the  $N \times M$  stoichiometry matrix and  $\mathbf{J} = \mathbf{J}_+ - \mathbf{J}_-$  is the  $M$ -dimensional flux vector with

$$J_+^j = k_+^j x_1^{\nu_1^j} x_2^{\nu_2^j} \dots x_N^{\nu_N^j} \quad \text{and} \quad J_-^j = k_-^j x_1^{\kappa_1^j} x_2^{\kappa_2^j} \dots x_N^{\kappa_N^j}$$

# Including External Fluxes

In the case of an open system which has external injection fluxes of species, for example:



we have:

$$\frac{dx_i}{dt} = \sum_{j=1}^M S_i^j J^j + J_i^e$$

which can be rearranged to give:

$$\frac{dx_i}{dt} = \sum_{j=1}^{\hat{M}} \hat{S}_i^j \hat{J}^j$$

# Stability of the Network

*“The key idea upon which the theory of stoichiometric dynamical systems is built is that in general the complete set of steady states can easily be calculated. It can always be represented parametrically in a simple form. Once this is done, any static or dynamical property that can be related to the steady states is ripe for investigation.”* - Bruce L. Clark

# Extensions to Ecology and Economics

The idea of stoichiometric modeling can be extended to many ecological and economic models as well, as long as the stoichiometries are real numbers and the reaction rates are proportional to continuous functions which are positive in the interior of the domain.

# For the General Model

For reactions in chemistry and interactions, birth processes, death processes, and immigrations in ecology:

$$\frac{dx_i}{dt} = \sum_{j=1}^M b_{ij} J_+^j - d_{ij} J_-^j$$

where the  $\mathbf{J}_+$ 's can be birth processes and  $\mathbf{J}_-$ 's can be death process, expressed as

$$J_+^j = k_+^j \prod_{i=1}^N x_i^{g_{ij}}$$

$$J_-^j = k_-^j \prod_{i=1}^N x_i^{h_{ij}}$$

# For Chemical Reaction Networks

We have certain relationships between the matrices:

$$\mathbf{B} = \mathbf{D} = \mathbf{H} - \mathbf{G}$$

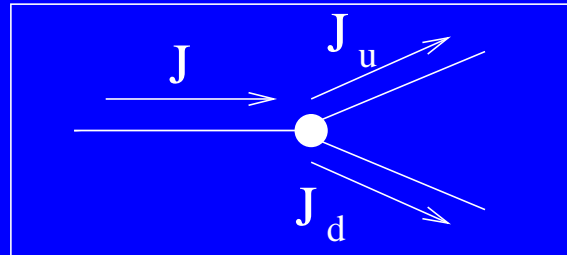
Equilibrium always exists with  $\mathbf{J}_+ = \mathbf{J}_- \geq \mathbf{0}$ . The linear stability is determined by the Jacobian matrix which can be shown to have all real, non-positive eigenvalues.

# Equilibrium vs. Non-equilibrium Steady-State

If there is no external flux, then the system of reactions is at equilibrium when  $\mathbf{SJ} = \mathbf{0}$ . However, if there are external fluxes acting on the system, then the system is at non-equilibrium steady-state (NESS) when  $\mathbf{SJ} = -\mathbf{J}^e$ .

# Kirchoff's Current Law and Flux Balance Analysis

Current Law: At any junction, the sum of the currents into that junction is zero.



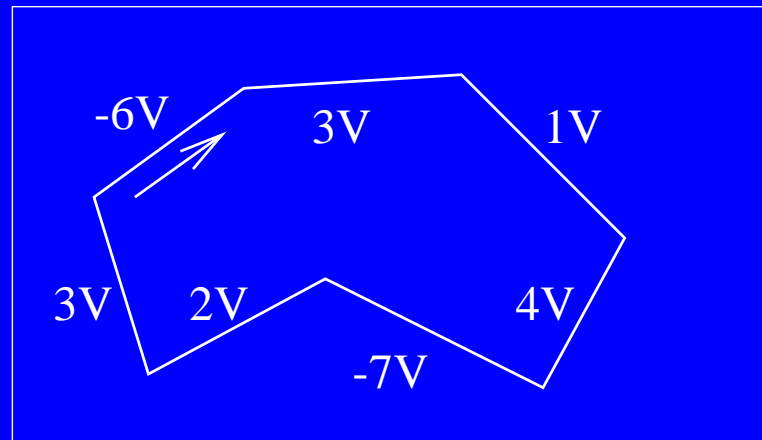
$$J = J_u + J_d$$

Any flux, **J**, which satisfies the equilibrium or NESS conditions satisfies the flux balance condition.



# Kirchoff's Voltage Law

Voltage Law: The change in potential, when summed around any loop within a circuit, is zero.



$$V_1 + V_2 + \cdots + V_N = 0$$

# Thermodynamics

If the chemical potential difference for each internal reaction is defined as  $\Delta G$  then from thermodynamics we know, for the sample reaction:



$$\Delta G = \Delta G^o + k_B T \ln \frac{c^{\alpha_3} d^{\alpha_4}}{a^{\alpha_1} b^{\alpha_2}}$$

and

$$\Delta G^o = -k_B T \ln \frac{k_+}{k_-}$$

Therefore,

$$\Delta G = k_B T \ln \frac{J_-}{J_+}$$

# Singular Value Decomposition

Consider now, only the internal reactions without the external fluxes. By using Singular Value Decomposition (SVD), we can decompose  $\mathbf{S}$  such that  $\mathbf{S} = \mathbf{D}\mathbf{\Sigma}\mathbf{B}^T$ .  $\mathbf{\Sigma}$  is the diagonal matrix containing the singular values of  $\mathbf{S}$  and has the form:

$$\mathbf{\Sigma} = \begin{pmatrix} \sigma_1 & \dots & 0 & 0 & \dots & 0 \\ \vdots & \ddots & \vdots & \vdots & \ddots & \vdots \\ 0 & \dots & \sigma_N & 0 & \dots & 0 \end{pmatrix},$$

the columns  $\mathbf{D}$  contain the left singular vectors of  $\mathbf{S}$ , and  $\mathbf{B}$  is the inverse of the matrix which has columns containing the right singular vectors of  $\mathbf{S}$ .

# Energy Balance Analysis

If  $\mathbf{S}$  has rank  $r$  then columns  $r + 1$  through  $M$  of  $\mathbf{B}$  span the null-space of  $\mathbf{S}$ . So we can define  $\mathbf{K}$  such that

$$\mathbf{K} = (\mathbf{B}(:, r + 1) \dots \mathbf{B}(:, M))$$

Then each row of  $\mathbf{K}$  provides the exact weights needed to balance the internal chemical reactions of the network.

# Energy Balance Analysis

Relating back to the thermodynamics, let us define the chemical potential of each reactant  $i$  as  $G_i$ . Then the chemical potential of each reaction is given by:

$$\sum_{i=1}^N G_i S_i^j = \Delta G^j$$

and multiplying by  $\mathbf{K}$  on the right we have:

$$G^T S K = \Delta G^T K = 0$$

which is a statement of global free energy balance for the network and is equivalent to Kirchhoff's voltage law.

# Second Law of Thermodynamics

The second law of thermodynamics requires that each reaction must dissipate energy. Therefore, in terms of  $J^j$ , which is the turnover per unit time, and  $\Delta G^j$ , which is the chemical potential change of turnover, we have:

$$-J^j \Delta G^j = -k_B T (J_+^j - J_-^j) \ln \frac{J_-^j}{J_+^j} \geq 0.$$

By summing over all reactions, this formula can be used to compute the total heat dissipation rate, which is necessarily equal to the entropy production rate of the isothermal biochemical network in a steady-state.